EXAMPLE VI

The Antimicrobial Effectiveness of Chlorine Dioxide in 0.02% Phosphate Solution

The following is an example of how to test the antimicrobial effectiveness of chlorine dioxide in 0.02% phosphate solution.

Materials

1. Purogene (2% chlorine dioxide), lot #8907:41, manufactured by BIO-CIDE International, Inc., P.O. Box 2700, Norman, Okla. 73070.
2. Test Organisms: Streptococcus mutans (ATCC #27152), Streptococcus sanguis (ATCC #10556), and Candida albicans (ATCC #18804)
3. Saline, 0.9% NaCl.
4. Butterfield's Buffer phosphate dilutent (BFB), pH 7.2.
5. Sterile 15% sodium thiosulfate.
6. Blood agar.
7. Stop watch.
8. Sterile 1N HCl and 1N NaOH.
9. pH meter.
10. McFarland nephelometer tube No. 1. Density of this tube is equivalent to a bacterial suspension of 3×10^8 organisms per ml.
11. N,N-diethyl-p-phenylenediamine (DPD reagent).
12. Phosphate buffer reagent.
13. Sodium dihydrogen phosphate, $NaH_2PO_4.7H_2O$. (Fisher Scientific, Fair Lawn, N.J.
14. Trisodium phosphate, $Na_3PO_4.12H_2O$. Albright & Wilson, P.O. Box 80, Oldbury, Narley, West Midlands, B694LN, England.
15. Sodium monofluorophosphate, Na_2FPO_3, Ref No. OB 12837, manufactured by Albright and Wilson, P.O. Box 80, Oldbury, Narley, West Midlands, B694LN, England.

DPD reagent and phosphate buffer reagent is prepared in accord with Standard Methods for the Examination of Water and Wastewater, 17th Edition, p. 9-54 (1989).

Methods

1. Test Solutions

A 0.2 percent sodium dihydrogen phosphate solution is prepared in distilled water. Ten ml is placed into each of five beakers. One of each of the five beakers receives 0, 1, 2.5, 5, and 10 ml of chlorine dioxide concentrate (2% ClO_2), respectively. All solutions are diluted to 90 ml with distilled water, adjusted to pH 6.0 with 1N NaOH and 1N HCl, diluted to 100 ml and placed in screw cap bottles. Solutions containing 0 ppm chlorine dioxide are filter sterilized prior to use.

Solutions containing trisodium phosphate and sodium monofluorophosphate are prepared in a similar manner.

II. Test Suspensions

Suspensions of each organism are prepared in Butterfield's buffer from 48 hour agar cultures and turbidity adjusted to a McFarland Tube #1. Subsequently 0.1 ml of this suspension is diluted in 50 ml of saline. The diluted microorganism suspensions are then ready for use.

III. Test Procedure

1. Test:

One ml of test suspension is aliquoted into each of five sterile 16×125 mm screw cap tubes. Each of the five tubes receives 4 ml of a solution containing either 0, 200, 500, 1000, or 2000 ppm chlorine dioxide in 1% sodium dihydrogen phosphate. Each tube is shaken for ten seconds and immediately inactivated with 0.25 ml 15% sodium thiosulfate. Solutions containing 1% trisodium phosphate and 1% sodium monofluorophosphate are handled in a similar manner.

2. Controls:

One ml of test suspension is dispensed into two sterile 16×125 mm screw cap tubes. Each tube receives 4 ml 2000 ppm chlorine dioxide in 0.02% sodium dihydrogen phosphate. The first tube receives 0.25 ml sodium thiosulfate, while the second tube receives none. Subsequently each tube is tested for residual chlorine dioxide by adding 0.3 ml phosphate buffer reagent and 0.3 ml DPD reagent to each tube. Neutralized tubes are colorless, while nonneutralized tubes are pink. Solutions of trisodium phosphate and sodium monofluorophosphate containing 2,000 ppm chlorine dioxide are handled in a similar manner.

One ml test suspension of each organism is treated with 4 ml Butterfield's buffer and 0.25 ml 10% sodium thiosulfate as a negative control.

After inactivation with sodium thiosulfate all tubes are plate counted.

Sterility tests on all reagents are run parallel to experiments by plate counted method. The plate counted method and sterility tests are conducted in accord with Standard Methods for the Examination of Water and Wastewater, 17th Edition, p. 9-54 (1989), in order to determine the antimicrobial effectiveness of chlorine dioxide in 0.02% phosphate solution.

It will be seen from the foregoing that the mixture of stabilized chlorine dioxide and phosphates as part of a mouth wash or toothpaste has an improved shelf life, and is effective as a bactericide superior to other compositions used today. Both stabilized chlorine dioxide, and phosphates, have been used for many years in other areas and extensive study in animals and in man have demonstrated the compounds' low toxicity and safety.

It will be obvious that various changes, alterations and modifications to the method and process described herein may be made, to the extent that such changes, alterations and modifications do not depart from the spirit and scope of the appended claims therein intended to be encompassed herein.

I claim:

1. A composition for preventing and treating dental disease by reducing the number of micro-organisms in the mouth, said composition comprising a non sudsing dentifrice, wherein the dentifrice contains stabilized chlorine dioxide in the concentration range of between about 0.005%-0.5% and a phosphate compound selected from the group consisting of disodium hydrogen phosphate, sodium dihydrogen phosphate, and trisodium phosphate in a concentration in the range of between about 0.02%-3.0% to retard escape of chlorine dioxide from said composition at a pH in the range of 6.0 to 7.4, thereby increasing the shelf life and efficacy of said composition.

2. The composition as set forth in claim **1**, wherein the concentration of stabilized chlorine dioxide is approximately 0.2% and the concentration of phosphate is approximately 1.0%.

3. A composition for preventing and treating dental disease by reducing the number of bacteria in the mouth, said composition comprising a non sudsing dentifrice, wherein the dentifrice contains at least 0.1% stabilized chlorine dioxide and at least 0.05% of a phosphate compound selected from the group consisting of